nature reviews endocrinology

https://doi.org/10.1038/s41574-025-01220-x

Review article

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Models of hyperglycaemia in diabetes mellitus and its complications

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Abstract

Diabetes mellitus is a metabolic disease marked by chronic high blood levels of glucose (also known as hyperglycaemia), which over time can cause damage to the blood vasculature, leading to devastating complications. The increase in global prevalence and the number of cases of type 2 diabetes mellitus continues unabated, prompting diabetes mellitus researchers to continue studying the aetiology of the disease in search of ways to improve therapeutic outcomes. Over the years, numerous in vivo and in vitro mammalian models have been developed to investigate the mechanisms underlying diabetes mellitus and its complications. The customary use or induction of high levels of glucose in vivo or in vitro to recapitulate chronic hyperglycaemia in diabetes mellitus is central to these models. However, there has never been a consensus as to how to standardize the re-creation of glucotoxicity conditions so that the pathophysiological effects in human diabetes mellitus can be accurately mimicked experimentally. In this Review, we critically discuss glucotoxicity (without lipotoxicity) in diabetes mellitus and analyse the various in vivo and in vitro models used to study the conditions created by high levels of glucose in diabetes mellitus. Efforts to standardize experimental induction or administration of high levels of glucose, and understanding the pros and cons of each model, could help harmonize findings that will benefit the diabetes mellitus research community.

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Key points

- Diabetes mellitus research relies on experimental models; both in vivo and in vitro mammalian models simulate hyperglycaemia to investigate glucotoxicity, β-cell failure and diabetes mellitus-related complications.
- Human intervention studies link excessive sugar intake to increased diabetes mellitus risk, but mechanistic effects on β-cell function and insulin sensitivity remain unclear due to confounding variables.
- Current models rely on arbitrarily defined 'acute' or 'chronic' hyperglycaemia, which might not reflect the progressive glucotoxicity occurring in human diabetes mellitus.
- In vivo rodent models can replicate diabetes mellitus and some complications, and reflect net systemic effects, but genetic background, obesity and inflammation complicate the isolation of glucose-specific effects.
- Controlled systems using pancreatic islets, β-cell lines and non-β-cell cell types allow detailed study of cellular responses to high levels of glucose and offer direct mechanistic insights.
- Future models should simulate moderate, fluctuating glucose levels for extended durations and include intercellular interactions to better reflect human diabetes mellitus pathophysiology.

Introduction

Diabetes mellitus is a global health crisis, currently affecting over 537 million people, with the number projected to rise to 784 million by 2045 (ref. 1). This heterogeneous condition encompasses monogenic diabetes mellitus, type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus. T1DM, an autoimmune disorder marked by β-cell destruction, accounts for roughly 10% of cases globally, whereas T2DM, which is characterized by β-cell dysfunction and insulin resistance, represents approximately 90% of cases globally. Diagnosis involves assessment of chronic hyperglycaemia through levels of HbA1c, fasting plasma levels of glucose or levels of glucose 2 h after a 75-g oral glucose tolerance test[2] (Supplementary Table 1).

Despite differences in aetiology, all types of diabetes mellitus share a defining feature: chronic hyperglycaemia. Thus, a core focus of diabetes mellitus research revolves around understanding how glucose homeostasis is regulated and disrupted. Under physiological conditions, glucose enters β-cells via GLUT transporters, initiating glycolysis and elevating the ratio of ATP to ADP. This change closes ATP-sensitive potassium channels, leading to membrane depolarization, opening of voltage-gated calcium channels and triggering calcium influx and insulin granule exocytosis. This glucose-stimulated insulin secretion (GSIS) is biphasic: an immediate release of prestored insulin, followed by a prolonged second phase involving new insulin synthesis[3]. Insulin then promotes glucose uptake in skeletal muscle, adipose tissue and liver, restoring normoglycaemia (Fig. 1).

In states of chronic nutrient excess and obesity, however, peripheral tissues such as muscle, adipose tissue and liver become progressively insulin-resistant[4]. To maintain euglycaemia, β-cells secrete more insulin and their numbers and/or size undergo compensatory increases. Although initially adaptive, this response imposes considerable metabolic and secretory stress, predisposing β-cells to exhaustion, insufficient compensation and a state referred to as glucotoxicity[5]. Chronic hyperglycaemia eventually triggers both microvascular complications such as diabetic retinopathy, diabetic kidney disease (DKD), diabetic foot ulcers (DFU) and neuropathy, and macrovascular complications such as stroke and diabetic cardiomyopathy, amplifying disease burden[6].

To study diabetes mellitus pathogenesis and related complications, diverse experimental models of hyperglycaemia in vivo and in vitro have been developed. Genetically modified animals, surgical interventions, chemical induction of diabetes mellitus and dietary manipulations in both humans and animals are all used to study the effects of hyperglycaemia. In parallel, in vitro models involve culturing metabolically relevant cells under varying levels of glucose to understand the direct cellular effects of hyperglycaemia. The following sections examine these models and their relevance in diabetes mellitus research.

Effects of high levels of glucose on human pathophysiology

Human intervention studies remain the most direct way to evaluate how high glucose intake affects metabolic health. Excessive consumption of sugar-sweetened and artificially sweetened beverages is linked to an increased diabetes mellitus risk, with each additional daily serving associated with higher incidence[7]. However, the literature presents inconsistent findings regarding the mechanistic effects of high sugar diets on insulin sensitivity and β-cell function (Table 1).

For example, one study found that individuals consuming more than one artificially sweetened soft drink per month had reduced β-cell glucose sensitivity and insulin secretion[8], whereas another study showed that participants fed 150 g of glucose or fructose per day for 4 weeks exhibited decreased insulin sensitivity[9]. Conversely, other studies found enhanced glucose metabolism: one demonstrated increased postprandial levels of glucose and insulin after a 55% carbohydrate diet[10] and another found increased insulin sensitivity after 5–14 days of a 75% carbohydrate diet, possibly as a compensatory glucose-clearing mechanism[11]. Yet, some studies found no substantial changes; for example, daily intake of 40 g or 80 g of sugar over 3 weeks did not alter fasting levels of glucose or insulin[12]. These inconsistencies highlight the limitations of human studies in controlling confounding variables and obtaining tissue-level mechanistic insights, which necessitate the use of experimental models.

In vivo models of hyperglycaemia in diabetes mellitus and its complications

Animal models remain indispensable for understanding the systemic consequences of chronic hyperglycaemia. These models simulate either insulin resistance, β-cell failure, or both, depending on the induction method: genetic, surgical, chemical (for example, alloxan or streptozotocin) or dietary manipulation (Supplementary Table 2).

This Review focuses on isolating hyperglycaemia-induced effects. In this context, two experimental variables, the magnitude and duration of elevated blood levels of glucose, govern model outcomes and dictate their relevance to different stages of diabetes mellitus pathophysiology. In the following sections, we summarize rodent models that recapitulate varying severities of hyperglycaemia and their utility for studying diabetes mellitus-related complications.

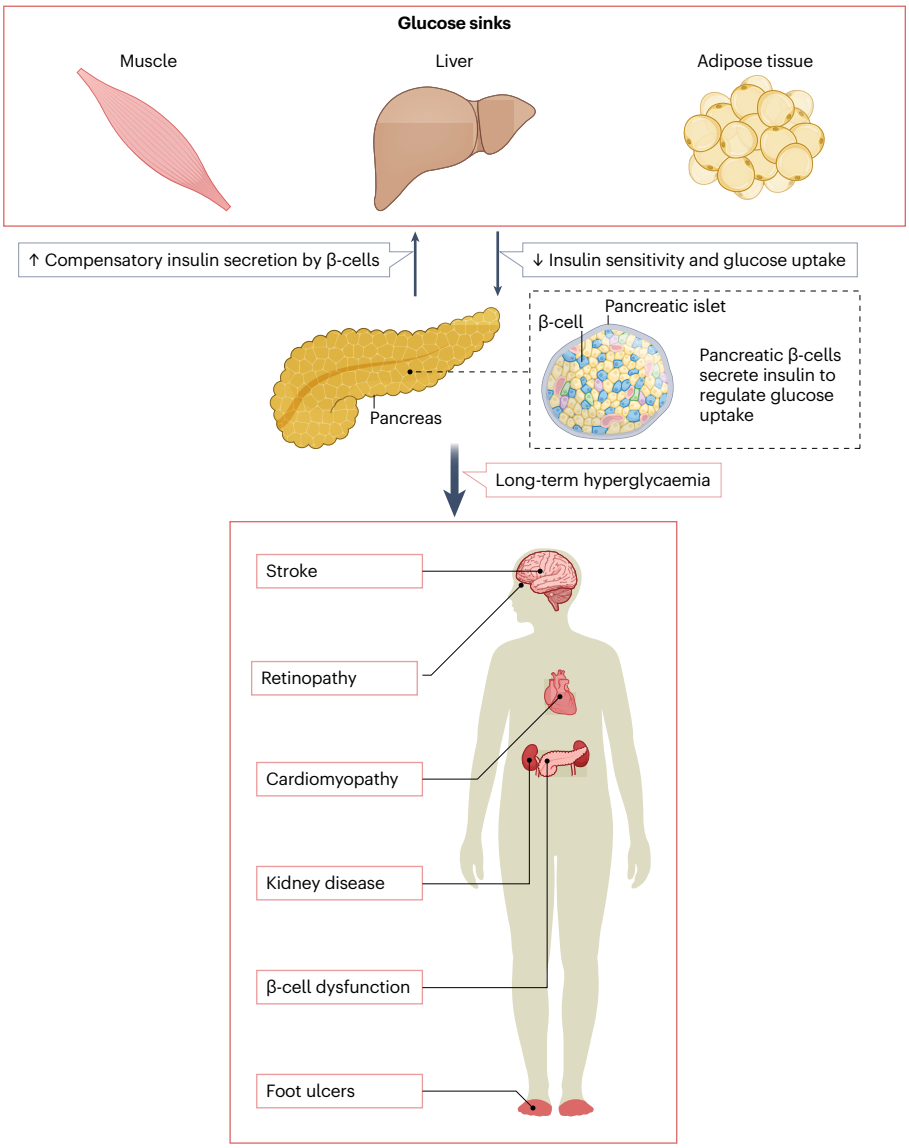

Fig. 1 | Dysregulation of insulin sensitivity, glucose uptake and pancreatic β-cell function leads to glucotoxicity and the development of diabetes mellitus-related complications. Various cells and tissues have roles in regulating blood levels of glucose, including pancreatic β-cells, which secrete insulin, and insulin-sensitive tissues such as the muscle, adipose tissue and liver cells. Long-term dysregulation within these tissues, if not adequately compensated, leads to glucotoxicity and subsequent microvascular and macrovascular complications associated with diabetes mellitus.

Genetic models of diabetes mellitus

Classic rodent models such as the *ob/ob* and *db/db* mice remain central to T2DM research. *ob/ob* mice lack leptin due to a *Lep* mutation and exhibit hyperphagia, obesity and β-cell dysfunction, while *db/db* mice have leptin receptor (*Lepr*) mutations that lead to defective signalling despite high levels of leptin. Both models develop severe insulin resistance and hyperglycaemia, ultimately progressing to β-cell failure. Similar phenotypes also occur in obese cats, dogs, pigs and non-human primates, but these models are beyond the scope of this Review[13].

Although these models emulate many features of human T2DM, the coexisting obesity, dyslipidaemia and inflammation complicate the isolation of hyperglycaemia-specific effects. Adipokine and cytokine signalling can confound interpretation of organ-specific glucose toxicity. To circumvent this limitation, non-obese diabetes models such as Akita mice are used. Their *Ins2* mutation causes proinsulin misfolding, endoplasmic reticulum (ER) stress and progressive β-cell apoptosis, leading to severe hyperglycaemia (>450 mg/dl) by 4 weeks of age without concomitant obesity or insulin resistance[14]. This model is valuable for investigating β-cell-intrinsic glucotoxicity, as the cells undergo considerable accelerated ER stress, apoptosis and insulin deficiency without peripheral insulin resistance[14].

Transgenic and knockout mouse models targeting insulin signalling or glucose transport pathways have also been utilized to dissect tissue-specific effects of hyperglycaemia, but often invoke compensatory responses, making it difficult to capture the multifactorial aetiology of diabetes mellitus. Moreover, hyperglycaemia in these models is often an indirect outcome of artificial gene modulation, rather than the product of naturally progressing disease.

Surgical models of diabetes mellitus

To achieve a more direct and adjustable method of inducing hyperglycaemia than genetic manipulation, surgical techniques such as partial

Table 1 | Summary of the effects of increased exposure to sugar on β-cell function in humans

Effect on human β-cell function	Finding	Ref.
Decrease	Increased consumption of artificially sweetened soft drink was associated with reduced β-cell glucose sensitivity and insulin secretion	8
	150 g of fructose or glucose for 4 weeks resulted in decreased insulin sensitivity	9
Increase	Increased carbohydrate consumption led to increased insulin secretion	10
	Increased carbohydrate consumption led to increased sensitivity to insulin in tissues	11
No change	Individuals who consumed 40 g or 80 g of sugar per day had no differences in fasting levels of glucose or insulin production	12

pancreatectomy are employed. This procedure, which removes 60–90% of the pancreas, has been performed in rodents, pigs and primates to reduce insulin-producing β-cell mass and induce hyperglycaemia[15–17]. It provides an opportunity to study β-cell regeneration and the consequences of insulin deficiency. However, this model is technically demanding and the compensatory regenerative capacity in rodents can result in variable glycaemic outcomes. In cases where compensation fails, severe hyperglycaemia ensues, limiting this technique's utility for studying gradual β-cell deterioration[18].

Chemical models of diabetes mellitus

Chemical induction methods offer an alternative, less invasive and more reproducible approach than surgical methods. The agents most commonly used are alloxan and streptozotocin, which selectively target pancreatic β-cells via GLUT2 transporters and induce oxidative stress, leading to cell death. These models are widely used to mimic T1DM-like conditions. Modified protocols using streptozotocin in combination with nicotinamide, a β-cell protective agent, have been used in rats to produce a T2DM-like phenotype. For example, intraperitoneal administration of 230 mg/kg nicotinamide followed by intravenous administration of 65 mg/kg streptozotocin induces partial β-cell destruction and moderate insulin deficiency without triggering autoimmunity[19,20]. Although promising, this protocol has not been widely validated in mice. To improve β-cell targeting, animals are typically fasted for 4–6 h prior to chemical administration to enhance GLUT2 expression and increase cellular uptake of streptozotocin or alloxan[21,22]. After injection, 10% sucrose in drinking water is often provided to prevent hypoglycaemia resulting from sudden insulin release by damaged β-cells. Nonetheless, these models typically generate abrupt and severe β-cell loss, which does not reflect the chronic, progressive nature of T2DM.

Diet-induced models of diabetes mellitus

To isolate hyperglycaemia from lipid-related complications, high-sugar diet models have been explored. Mice provided with 10% glucose water for 12 weeks show only modest glucose intolerance and no weight gain or insulin resistance[23]. Conversely, 74% starch diets cause progressive hyperglycaemia and β-cell hypertrophy by week 15 (ref. 24), whereas 60% fructose diets lead to glucose intolerance within 4–8 weeks[25]. However, studies in humans have suggested that fructose might have different effects on glycaemic control in humans than in mice[26].

Collectively, genetic, surgical, chemical and dietary models each provide distinct insights into the pathogenesis of diabetes mellitus and the progression of hyperglycaemia. Although no single model perfectly replicates human disease, their complementary nature enables researchers to investigate various aspects of β-cell failure, insulin resistance and systemic glucotoxicity. Persistent hyperglycaemia modelled in these systems can also contribute to secondary complications such as retinopathy, nephropathy, neuropathy and cardiomyopathy. A comprehensive understanding arises from integrating findings across models to guide targeted interventions. Researchers must use these models not only to study glucose homeostasis but also to dissect the organ-specific consequences of hyperglycaemia.

In vivo models of hyperglycaemia-driven diabetes mellitus-related complications

In the context of persistent hyperglycaemia as the principal driver of diabetes mellitus-related complications, this section focuses on glucose-induced effects rather than on genetic models that directly impair organ function (Table 2). We examine how the duration and intensity of hyperglycaemia affect the onset and progression of complications such as diabetic retinopathy, DKD, DFU, diabetic peripheral neuropathy (DPN) and diabetic cardiomyopathy in rodent models (Fig. 2), with a focus on their relevance to human disease.

In humans, diabetic retinopathy primarily manifests as vascular damage, whereas rodent models encompass vascular, neural and visual deficits[27,28]. Streptozotocin-induced diabetic mice show considerable variation in diabetic retinopathy onset, ranging from 1 to 44 weeks, with most developing symptoms around 8 weeks after induction[29–32]. By contrast, *db/db* mice consistently exhibit early diabetic retinopathy within 2–4 weeks of hyperglycaemia onset[33,34]. Persistent hyperglycaemia induces diabetic retinopathy through pro-inflammatory signalling[35], leukocyte activation[36] and accumulation of advanced glycation end products, which impair capillary function[37,38]. Additionally, PKC activation and reactive oxygen species (ROS) generation increase retinal apoptosis and vascular permeability[39,40]. The earlier diabetic retinopathy onset in *db/db* mice suggests that obesity-associated inflammation might exacerbate damage[41]. However, most rodent models only mimic early diabetic retinopathy stages and lack proliferative features such as neovascularization. The Akimba mouse, which combines the Akita and Kimba models, was created to address this gap but relies on VEGF overexpression, which limits its specificity to hyperglycaemia[42–44].

In DKD models, the duration and genetic background influence disease severity. Streptozotocin-induced diabetes mellitus in DBA/2 and BALB/c mice produces albuminuria after 8 weeks, while other strains show milder disease[45]. After 25 weeks of hyperglycaemia, DBA/2J mice and KK/HIJ mice display notable renal pathology despite similar levels of glucose to resistant strains[46], indicating a strong role for genetic predisposition. In *db/db* mice, albuminuria begins by 8 weeks and progresses to severe DKD by 25 weeks[47].

Delayed wound healing is another consequence of hyperglycaemia. DFU models using streptozotocin, alloxan or Akita mice show impaired healing within 15 days, with *db/db* mice show the slowest healing due to obesity-related inflammation and poor vascularization[48]. Hyperglycaemia duration also affects DPN. Streptozotocin models show strain-dependent dystrophy within 2–3 months in DBA/2J mice, 2–5 months in C57BL/6 mice and 5–12 months in B6D2F1 mice[49]. Interestingly, despite similar levels of hyperglycaemia, *db/db* mice rarely develop neuritic dystrophy, suggesting genetic protection.

While no rodent model has precisely defined the timeline of hyperglycaemia-induced stroke, diabetes mellitus is a known risk factor for stroke in humans[50]. Mice with streptozotocin-induced diabetes mellitus in which intracerebral haemorrhage is induced display worse outcomes than non-diabetic mice with intracerebral haemorrhage, with larger haematomas and increased oxidative stress[51]. Diabetic cardiomyopathy remains inconsistently modelled. Streptozotocin-treated mice and Akita mice rarely develop notable cardiac dysfunction[52,53], whereas *db/db* and *ob/ob* mice show hypertrophy and contractile impairment by 12 weeks[54,55], probably due to leptin deficiency affecting cardioprotection[56].

Although rodent models provide systemic exposure to chronic hyperglycaemia, interspecies physiological differences limit their translatability[57,58]. When induction fails to reproduce complications, genetic manipulation is often employed, potentially confounding efforts to isolate glucose-specific effects.

Limitations of in vivo models of diabetes mellitus

In vivo models have long served as essential tools in diabetes mellitus research, offering the ability to study how chronic hyperglycaemia contributes to systemic and organ-specific complications such as diabetic retinopathy, nephropathy and neuropathy. These models recreate a physiologically relevant environment where multiple organ systems are simultaneously exposed to high levels of glucose, making them well-suited for investigating disease progression in a whole-organism context. However, despite their strengths, in vivo models present considerable limitations that constrain their ability to isolate the direct effects of hyperglycaemia.

A primary limitation lies in the variability in the onset, duration and severity of hyperglycaemia across different in vivo models. Whether induced genetically, surgically, chemically or through diet, blood levels of glucose can vary substantially between animals, strains and experimental conditions. This inconsistency compromises reproducibility and complicates interpretation. Moreover, systemic factors such as obesity, chronic inflammation and dyslipidaemia frequently coexist in these models, potentially masking the specific effects of hyperglycaemia on individual tissues. While these factors reflect the multifaceted nature of diabetes mellitus, they pose challenges when the aim is to disentangle glucose-specific pathology.

It is important to note, however, that the exclusion of lipid-related perturbations represents both a strength and a limitation of hyperglycaemia-focused models. In T2DM, hyperglycaemia rarely occurs in isolation. It develops in the context of obesity, hyperinsulinaemia, and elevated levels of circulating lipids, which together exacerbate lipotoxic stress and glucolipotoxic stress on metabolic tissues[59]. Separating lipid-driven components allows clearer attribution of observed phenotypes to glucose excess but can underrepresent the integrated metabolic stress characteristic of human T2DM. Studies that combine controlled manipulation of levels of glucose and lipids will therefore be essential to recapitulate the full pathophysiological spectrum of diabetic dysfunction. When glucose regulation fails, impaired cellular glucose uptake drives a metabolic shift towards fatty acid oxidation, leading to excessive production of acetyl-CoA and ketone bodies such as acetoacetate and β-hydroxybutyrate[60]. This dysregulated lipid metabolism further acidifies the systemic milieu and exacerbates cellular stress, compounding the pathological effects of chronic hyperglycaemia.

Another drawback is limited translatability to human diabetes mellitus. Rodent models often fail to replicate the chronic and heterogeneous course of human diabetes mellitus, particularly for late-stage complications such as advanced kidney disease or diabetic

Table 2 | In vivo models of hyperglycaemia-driven diabetes mellitus giving rise to complications

Complication	Mouse models	Hyperglycaemia duration (weeks)	Blood level of glucose (mg/dl)	Ref.
Diabetic retinopathy	Streptozotocin treatment	8	Unknown	32
	Streptozotocin treatment	8	Unknown	104
	Streptozotocin treatment	8	Unknown	105
	db/db mice	4	200	33
	db/db mice	4	400–500	106
Diabetic kidney disease	Streptozotocin treatment in DBA/2 mice	8	400–500	45
	Streptozotocin treatment in DBA/2J mice	25	450–600	46
	Streptozotocin treatment in BALB/c mice	8	150–200	45
	Streptozotocin treatment in129/SvEv mice	8	180–220	45
	Streptozotocin treatment in MRL/MpJ mice	8	150–200	45
	Streptozotocin treatment in MRL/MpJ mice	25	380–470	46
	Streptozotocin treatment in C57BL/6J mice	8	500–550	45
	Streptozotocin treatment in C57BL/6J mice	25	450–550	46
	Streptozotocin treatment in KK/HIJ mice	25	300–360	
	Streptozotocin treatment in A/J mice	25	350–450	
Diabetic peripheral neuropathy	NOD mice	3–5	>600	49
	Streptozotocin treatment in DAB/2 J mice	8–12	500–550	
	Streptozotocin treatment in C57BL/6J mice	8–20	350–450	
	Streptozotocin treatment in B6D2F1 mice	20–48	480–550	

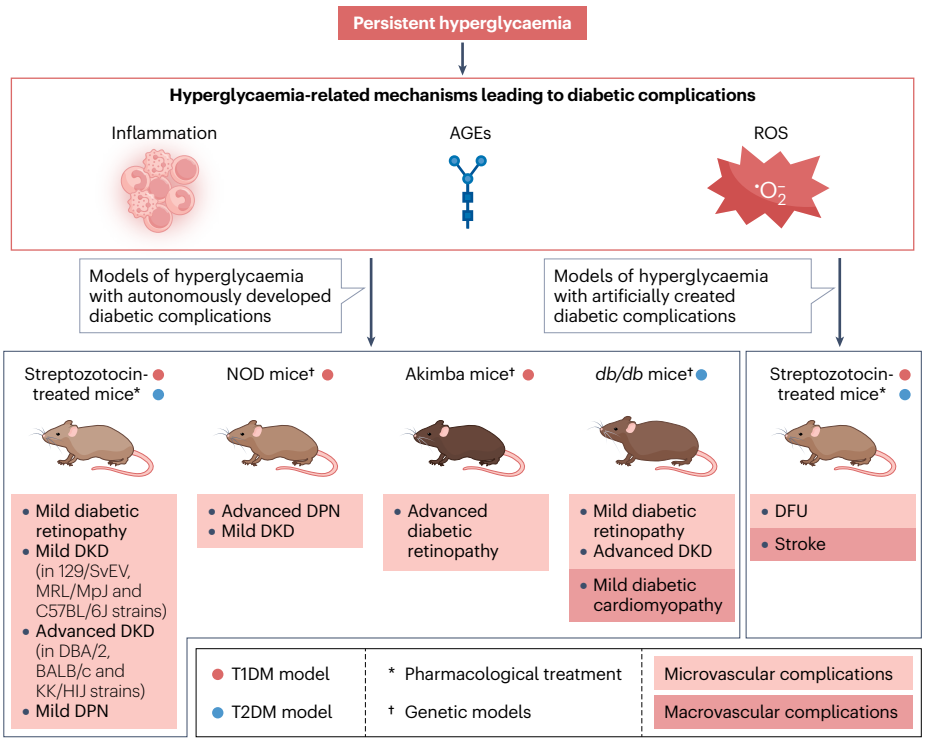

Fig. 2 | Mouse models of diabetes mellitus-related complications arising from persistent hyperglycaemia. Persistent hyperglycaemia is a central driver of complications in type 1 diabetes mellitus (T1DM) and type 2 diabetes (T2DM) mouse models. Hyperglycaemia increases levels of inflammation, advanced glycation end-products (AGEs) and reactive oxygen species (ROS), which contribute to organ-specific dysfunction. There is a spectrum of complications observed in different mouse models: streptozotocin-treated mice exhibit mild diabetic retinopathy, mild and advanced diabetic kidney disease (DKD) and mild diabetic peripheral neuropathy (DPN). Diabetic foot ulcers (DFU) and stroke do not develop due to streptozotocin treatment alone, but can be induced in streptozotocin-treated mice to model how these conditions affect people with diabetes mellitus. NOD mice develop advanced DPN and mild DKD; Akimba mice demonstrate advanced diabetic retinopathy; and *db/db* mice display mild diabetic retinopathy, advanced DKD and mild diabetic cardiomyopathy. This representation underscores the pathophysiological links between persistent hyperglycaemia and the progression of microvascular and macrovascular complications in diabetes mellitus. 'Autonomously developed diabetic complications' refers to complications that arise due to the genetic or pharmacological intervention that induces diabetes mellitus in these models. 'Artificially created diabetic complications' refers to complications that do not develop in these rodent models without additional procedures.

cardiomyopathy. Temporal glucose fluctuations in rodent models, ranging from brief spikes to persistent elevations, rarely align with the complex and fluctuating patterns observed in human patients. These differences reduce the predictive power of in vivo systems, especially for evaluating therapeutic interventions or understanding long-term cellular stress responses to glucose.

To overcome these constraints, researchers frequently employ in vitro systems as complementary platforms. These models allow precise control over levels of glucose, exposure duration and other environmental variables, providing a more defined setting for investigating cellular responses to hyperglycaemia. Free from systemic confounders, in vitro models facilitate the direct study of molecular and metabolic changes triggered by high levels of glucose in specific cell types. As such, they offer valuable insights into the mechanistic underpinnings of glucose-induced dysfunction and could serve as a foundation for developing more targeted therapies.

In summary, although in vivo models remain indispensable for studying diabetes mellitus in a systemic context, their limitations highlight the importance of integrating in vitro systems for mechanistic precision and translational relevance.

In vitro models of hyperglycaemia in diabetes mellitus and its complications

To address the limitations discussed in the preceding section, in vitro models serve as critical complementary tools. These models offer controlled environments to isolate and examine the cellular and molecular mechanisms that underlie the pathophysiological effects of hyperglycaemia, independent of systemic variables. By enabling precise manipulation of concentrations of glucose and exposure duration, in vitro studies allow researchers to dissect glucose-induced stress responses at a high temporal and spatial resolution. In this section, we focus on two main classes of in vitro models: isolated pancreatic islets and β-cell lines, followed in subsequent sections by other non-β-cell models (Table 3) relevant to diabetes mellitus-related complications.

Exposure of islets to different concentrations and durations of hyperglycaemia

Rodent islets are commonly used for their accessibility, although human islets provide more translational value. Short-term exposure of rodent islets to 16.7 mM glucose for 24 h initially triggered enhancement in insulin secretion, which was attributed to increased

intracellular levels of calcium and exocytotic readiness. Capacitance measurements and delayed repolarization confirmed enhanced depolarization under these conditions, reflecting an acute adaptive response to hyperglycaemia[61]. However, prolonged exposure quickly led to β-cell dysfunction. Rodent islets exposed to 16.7 mM glucose for 48 h showed impaired oxidative metabolism driven by mTORC1 activation and phosphorylation of pyruvate dehydrogenase by PDK1. This impairment restricted pyruvate entry into the tricarboxylic acid cycle, reducing ATP production and impairing insulin secretion[62,63]. By 4 days, islets showed blunted GSIS and a reduced stimulation index, indicating a loss in glucose-sensing fidelity[64].

Human islets often exhibit a more rapid decline under hyperglycaemic conditions. Exposure to 11 mM glucose for 96 h reduced insulin stores and disrupted secretion rhythms, although co-treatment with ATP-sensitive potassium channel openers partially restored function[65]. Human islets exposed to 28 mM glucose for 7 days showed pronounced GSIS defects, reduced insulin content and elevated proinsulin accumulation, which are hallmarks of ER stress and impaired insulin processing[66,67]. These findings highlight both conserved and species-specific vulnerabilities to hyperglycaemia.

Exposure of β-cell lines to various concentrations and durations of hyperglycaemia

Studies using immortalized β-cell lines provide complementary mechanistic insights. Common models include mouse MIN6, rat INS-1E and human EndoC-βH lines[68–71]. MIN6 cells exposed to 25 mM glucose for 24 h showed reduced insulin secretion and downregulation of *Slc2a2* (which encodes GLUT2), similar to transcriptomic profiles in pancreatectomized rats[64]. Long-term exposure to high levels of glucose further impaired MIN6 cell function, causing dedifferentiation and increased proliferation[24]. Similarly, INS-1E cells exposed to 25 mM glucose for 48 h showed reduced GSIS, increased glycolytic flux, mTORC1

Table 3 | In vitro models of hyperglycaemia in β-cell lines and non-β-cell lines

Organ or cell type of interest	Model cell or tissue type	Concentration of glucose (mM)	Exposure duration	Refs.
Pancreas	Rodent islets	16.7	24 h	61
	Rodent islets	16.7	48 h	62,63
	Rodent islets	16.7	4 days	64
	Human islets	11	96 h	65
	Human islets	28	7 days	66,67
	MIN6 cells	25	24 h	64
	INS-1E cells	25	48 h	63
	EndoC-βH3 cells	15–30	3 weeks	72
Skeletal muscle	Mouse satellite cells	19	4 days	73
	Human skeletal muscle myotubes	20	4 days	74
Adipocytes	3T3-L1 mouse adipocytes	25	18 h	76
	3T3-L1 mouse adipocytes	25	7 days	77
	3T3-L1 mouse adipocytes	25	3 weeks	78
Liver	Rat hepatoma Fao cells	20	24 h	79
	Rat hepatocytes	20	24 h	80
Eye	Human MIO-M1 Müller cells	25	72 h	81
	Human retinal endothelial cells	25	72 h	82
Kidney	Rabbit proximal tubular cells	25	24 h	90
	Human HK-2 renal cells	25	24 h	91
	Human renal tubular cells	25	6 days	92
Neurons	Mouse dorsal root ganglion neurons	25	24 h	83
	Human astrocytes	25	3 days	87
	Rat Schwann cells	25	14 days	88
Blood vessels	Human umbilical vein endothelial cells	25	24 h	93
	Human aortic smooth muscle cells	25	24 h	94
	Human aortal endothelial cells	25	48 h	95
Cardiomyocytes	Induced pluripotent stem cell-derived cardiomyocytes	25	24 h	98
	Neonatal rat cardiomyocytes	25.5	48 h	99
	Cardiac stem cells	25	4 weeks	100

activation, AMPK suppression and elevated levels of caspase 3, indicating apoptosis[63]. These responses mirror in vivo β-cell failure pathways, validating their relevance for mechanistic studies. EndoC-βH3 cells exposed to 15–30 mM glucose for 3 weeks showed upregulation of insulin processing and proliferation genes such as *BIRC5* and *EGR1* (ref. 72). However, the lack of reported GSIS limits interpretation. Given that β-cells from adult humans have low proliferative potential, the relevance of these changes to in vivo physiology remains uncertain.

In contrast to islets, β-cell lines often fail to show early adaptive responses to glucose. For example, MIN6 cells exposed to 25 mM glucose for 24 h lack the transient insulin secretion increase seen in intact islets, probably due to the absence of intra-islet paracrine signalling. This observation suggests that β-cell lines are more prone to rapid dysfunction and might represent later disease stages.

Together, findings from islet models and β-cell models illustrate the progressive transition from adaptation to maladaptation under chronic hyperglycaemia. Initially, elevated levels of glucose enhance oxidative metabolism, ATP production and insulin secretion in an adaptive phase that sustains glucose clearance. Over time, however, this adaptation becomes maladaptive, characterized by mitochondrial dysfunction, excessive ROS generation, ER stress, mTORC1 hyperactivation and suppression of AMPK signalling, all of which converge to impair insulin synthesis and secretion. Prolonged metabolic stress further induces β-cell dedifferentiation, loss of identity and apoptosis, culminating in irreversible β-cell failure.

In summary, in vitro studies using islets and β-cell lines have revealed critical temporal and mechanistic insights into the adaptive and maladaptive responses of β-cells to hyperglycaemia. Although rodent models provide valuable mechanistic entry points, human-derived systems offer better translational relevance. Future studies integrating chronic moderate glucose exposure, species-specific insights and multiomics profiling will be key to understanding β-cell failure in diabetes mellitus and developing targeted therapies.

Exposure of non-β-cells to high levels of glucose

Although studies using β-cell lines have advanced our understanding of cellular responses to hyperglycaemia, they capture only part of the broader landscape of diabetes mellitus-related dysfunction. Non-β-cell models, spanning skeletal muscle, adipose tissue, liver, retina, neurons, renal cells, vascular cells and cardiomyocytes, reveal tissue-specific pathologies triggered by high levels of glucose. These in vitro systems provide mechanistic insights into how chronic hyperglycaemia drives systemic metabolic derangements and organ-specific complications.

In skeletal muscle, hyperglycaemia impairs both metabolism and regenerative capacity. Mouse satellite cells exposed to 19 mM glucose for 4 days showed reduced proliferation and $Pax7^+$ reserve cells, suggesting impaired regeneration[73]. Human myotubes exposed to 20 mM glucose exhibited reduced insulin-stimulated glucose uptake and glycogen synthesis, with unchanged glucose oxidation and increased lipogenesis[74]. Similarly, induced pluripotent stem cell (iPSC)-derived myoblasts exposed to 5 mM, 17.5 mM or 25 mM glucose displayed glucose-dependent proliferative impairment, with hyperglycaemic myoblasts showing cell cycle arrest at the S phase and at the transition from the G2 phase to the M phase and mitochondrial dysfunction. Upon terminal differentiation, these cells formed hypertrophic and highly branched myotubes with disorganized myosin heavy chain structures and increased fatiguability, indicating impaired regenerative and contractile function characteristic of diabetic myopathy[75]. These effects might contribute to sarcopenia and whole-body insulin resistance in diabetes mellitus.

Adipocytes also exhibit time-dependent dysfunction. Exposure of 3T3-L1 adipocytes to 25 mM glucose for 18 h reduced GLUT4 translocation, disrupting glucose uptake[76]. After 7 days, cells became hypertrophic, with increased NF-κB signalling and downregulation of PPARγ, accompanied by elevated levels of SAA3 and hyaluronan, which are markers of chronic inflammation[77]. At 3 weeks, adipogenesis and lipid accumulation accelerated, with a 1.7-fold increase in cell size[78]. These results illustrate how sustained hyperglycaemia promotes insulin resistance and adipose tissue inflammation.

In hepatic cell models, hyperglycaemia alters glucose handling. Fao cells exposed to 20 mM glucose for 24 h exhibited a 40% rise in gluconeogenesis via elevated PEPCK expression, driven by an increased ratio of liver activating protein, which is an active isoform of C/EBPβ, to an inhibitory isoform of C/EBPβ known as liver inhibitory protein[79]. Similarly, rat hepatocytes exposed to 20 mM glucose showed increased glycogen storage and lactate output, reflecting adaptive but inefficient metabolism[80].

Retinal cells provide insights into early diabetic retinopathy. Human MIO-M1 Müller cells exposed to 25 mM glucose for 72 h showed upregulation of RAGE and S100B, stimulating MAPK-driven inflammation[81]. Endothelial tight junction proteins such as claudin 5 and occludin were lost under similar conditions, increasing permeability[82]. Long-term exposure led to endothelial senescence and glycolytic dysfunction.

Neurons and glial cells respond to hyperglycaemia with structural and metabolic alterations. Embryonic chick dorsal root ganglia neurons exposed to 25 mM glucose for 24 h exhibited impaired neurite outgrowth, increased caspase 3 and caspase 7 activity and LDH release, indicating oxidative stress-induced apoptosis[83]. Similarly, iPSC-derived neural stem cells exposed to high levels of glucose (25 mM) displayed attenuated proliferation and reduced βIII-tubulin expression during neuronal differentiation for 7 days, leading to shortened neurite networks and impaired neuronal connectivity. The effect was accompanied by increased caspase 1 expression, indicating inflammatory activation mediated by high levels of glucose and suggesting a mechanistic basis for maternal diabetes mellitus-related neural tube defects[84]. In parallel, iPSC-derived neurons exposed to high levels of glucose exhibited decreased expression of the retromer component VPS26A due to ROS–NF-κB–DNMT1-mediated promoter hypermethylation[85]. This retromer dysfunction led to amyloid precursor protein retention, increased levels of amyloid β and phosphorylated tau and synaptic deficits, mirroring early Alzheimer-like pathology in diabetic models. In diabetic mice, VPS26A restoration ameliorated cognitive decline. 3T3-HIR cells exposed to 25 mM glucose for 48 h showed enhanced insulin receptor expression and glycolytic activity[86]. In astrocytes, exposure to 25 mM glucose for 3 days triggered cell cycle arrest and elevated levels of ATP, glycogen and AMPK signalling[87]. Schwann cells exposed to high levels of glucose for 14 days showed abnormal proliferation, extracellular matrix protein expression and p38 MAPK activation, which are hallmarks of early diabetic neuropathy[88,89].

Renal tubular cells exhibit oxidative and metabolic stress when exposed to high levels of glucose. In proximal tubule cells, exposure to 25 mM glucose for 24 h triggered NADPH oxidase activation, mitochondrial dysfunction and decreased antioxidant defences[90]. HK-2 cells under similar conditions displayed elevated ratios of lactate to pyruvate and reduced levels of tricarboxylic acid cycle intermediates[91].

By day 6, increased thymidine uptake and collagen synthesis observed in proximal tubule cells indicate early fibrotic changes[92].

Dysfunction develops rapidly in vascular cells exposed to high levels of glucose. In human umbilical vein endothelial cells, exposure to 25 mM glucose for 24 h impaired PI3K–AKT–eNOS signalling while upregulating ERK1 and ERK2, reducing nitric oxide production[93]. In aortic smooth muscle cells, oxidative stress increased via NADPH oxidase[94]. In human aortal endothelial cells, exposure to high levels of glucose for 48 h raised the levels of methylglyoxal and triggered the unfolded protein response, promoting inflammation and protein synthesis defects[95].

Cardiomyocytes exposed to 25 mM glucose show progressive remodelling. HL-1 cells exhibit increased lactate production, MMP loss and increased levels of mitochondrial ROS after 12–72 h[96]. Rat cardiomyocytes show enhanced protein synthesis via PI3K–AKT and MAPK activation[97]. In iPSC-derived cardiomyocytes, mitochondrial fission and DRP1 upregulation were observed after 24 h[98]. In neonatal rat cardiomyocytes, hyperglycaemia induced hypertrophy via PKC–NF-κB–c-fos signalling[99] and exposure of cardiac stem cells to high levels of glucose for 4 weeks reduced viability and angiogenic capacity[100]. In human iPSC-derived cardiomyocytes, exposure to high levels of glucose (15 mM) prolonged field potential duration (FPD) and corrected FPD (FPDc), increased beating rate and heightened arrhythmogenic susceptibility, mimicking QT prolongation observed clinically in diabetes mellitus. Insulin co-treatment ameliorated these abnormalities, reducing the prolongation of FPD and FPDc and arrhythmia risk. Additionally, lipotoxicity induced by high levels of glucose, characterized by lipid accumulation, impaired calcium handling and cellular hypertrophy occurred. These effects were mitigated by the SGLT2 inhibitor empagliflozin, which normalized calcium transients and contractile function[101,102].

Collectively, these non-β-cell models demonstrate how hyperglycaemia induces progressive dysfunction across multiple organs, providing mechanistic frameworks for understanding diabetic complications.

Limitations of in vitro models of diabetes mellitus-related complications

In vitro models offer controlled and reproducible systems to study the cellular and molecular mechanisms underlying diabetes mellitus and its complications, allowing precise manipulation of concentrations of glucose and exposure durations. Such models are essential for dissecting hyperglycaemia-specific responses, free from the systemic confounders inherent in in vivo systems. For instance, rodent and human islets exposed acutely (24–48 h) or chronically (96 h to 7 days) to high levels of glucose have been instrumental in identifying β-cell responses, including an initial increase in insulin secretion followed by dysfunction marked by oxidative stress, metabolic inflexibility and impaired glucose sensing. Similarly, β-cell lines such as MIN6, INS-1E and EndoC-βH provide platforms to explore gene expression, insulin secretion and apoptosis in response to hyperglycaemia under highly reproducible conditions.

Despite their advantages, in vitro models face inherent limitations in mimicking the complexity and temporal dynamics of hyperglycaemia observed in patients with diabetes mellitus. Most in vitro experiments expose cells to sustained, supraphysiological glucose concentrations (for example, 25 mM), which can exaggerate stress responses and obscure the slower, progressive decline in function seen in vivo. Moreover, isolated β-cell cultures lack paracrine and vascular interactions, limiting their ability to capture the multicellular crosstalk critical for β-cell adaptation. This limitation is particularly evident in β-cell lines, where the absence of paracrine interactions accelerates stress and skips over early adaptive responses observed in intact islets.

Another limitation arises from species differences. Rodent islets, although more abundant and experimentally convenient, are more resistant to hyperglycaemia-induced dysfunction than human islets, limiting their translational relevance. Human islets, though more representative of disease progression, are costly and limited in supply, making them impractical for large-scale studies. Additionally, β-cell lines, while consistent and convenient, lose insulin secretion capacity after long-term culture or multiple passages, making them suboptimal for long-term studies on chronic hyperglycaemia.

Beyond β-cells, in vitro models derived from non-β-cell tissues such as skeletal muscle, adipocytes, liver, kidney, retina and vasculature provide valuable insights into the tissue-specific complications of diabetes mellitus. These models enable researchers to isolate the direct effects of glucose on cell function, such as impaired glucose uptake in skeletal muscle or enhanced oxidative stress in renal tubular cells. However, without systemic crosstalk, these models cannot fully replicate how hyperglycaemia-driven dysfunction spreads across organ systems. The concentrations of glucose and exposure durations used in these models also considerably influence outcomes; short-term exposures might highlight acute stress responses, whereas long-term exposure reveals progressive dysfunction. Thus, careful calibration of these variables is essential to ensure experimental relevance to human disease.

In summary, although in vitro models are indispensable for mechanistic studies of hyperglycaemia, they cannot fully replicate the systemic and multifactorial nature of diabetes mellitus. Their value lies in the ability to isolate and study specific responses under tightly controlled conditions. However, translating these findings into meaningful conclusions about disease progression requires integration with in vivo and clinical data. Together, these complementary approaches can advance our understanding of how chronic glucose elevations disrupt metabolic homeostasis and promote organ dysfunction.

Advancing models of hyperglycaemia: a call to action

The field of diabetes mellitus research stands at a critical juncture where refining models of hyperglycaemia could transform our understanding of the disease. Existing in vivo and in vitro systems have yielded foundational insights, but often fall short in recapitulating the progressive, multifactorial nature of human diabetes mellitus. There is a growing need for physiologically relevant models that better simulate real-world glucose dynamics, tissue interactions and systemic responses.

A key area for improvement lies in developing models that mimic moderate, long-term hyperglycaemia with the variability observed in patients. Many current studies rely on acute or excessive glucose exposure, which can trigger artificial stress responses and miss subtler pathways implicated in disease progression. Incorporating dynamic glucose control systems, co-culture platforms and organ-on-chip technologies[103] could help bridge this gap. Moreover, humanized models such as β-cell xenografts in immunodeficient mice could integrate paracrine signalling and inter-organ interactions that are critical to the pathogenesis of diabetes mellitus.

Improving these models also has implications for public health messaging. Although reducing sugar intake is widely promoted as a preventive measure, this singular focus oversimplifies diabetes mellitus as a disease of excess sugar. In reality, diabetes mellitus arises

from a complex interplay of genetics, environmental factors, insulin resistance and β-cell dysfunction. By emphasizing metabolic health over sugar alone, researchers and clinicians can adopt a more comprehensive approach to prevention and treatment.

Ultimately, developing better hyperglycaemia models is not just a technical imperative but a necessary step towards understanding the disease in all its dimensions. Such models will help identify novel therapeutic targets, validate interventions aimed at mitigating glucose variability and inform more nuanced strategies for managing diabetes mellitus. Embracing the complexity of diabetes mellitus will allow the scientific and public health communities to move beyond oversimplification and towards more effective solutions.

Conclusions

Overall, models for diabetes mellitus that use high levels of glucose simulate the effects of sustained high blood levels of glucose on the body. However, the various in vivo and in vitro models have limitations in fully capturing all the damage caused by hyperglycaemia. We compare the advantages and disadvantages of these different in vivo and in vitro models used in modelling diabetes mellitus. There remains the need to critically evaluate how relevant these models are to diabetes mellitus in humans, in a defined and contextualized manner. Where possible, newer and more relevant models of diabetes mellitus in which the concentration and duration of exposure to high levels of glucose more accurately reflect diabetes mellitus in humans are needed. Therefore, a continued critical evaluation of how exposure to high levels of glucose can mimic the detrimental effects in diabetes mellitus can lead to a better understanding of diabetes mellitus and the development of future treatment strategies.

Published online: 06 January 2026

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Acknowledgements

The authors thank members of the Teo laboratory for the critical reading of this manuscript. R.Y.H.L. is supported by the National University of Singapore (NUS) Yong Loo Lin School of Medicine Graduate Studies Award (GSA). X.Z. is supported by the NUS Research Scholarship (RS). C.C. is supported by the Precision Medicine (PM) Translational Research Programme (TRP) Ph.D. Scholarship. A.K.K.T. is supported by IMCB, A*STAR, HLTRP/2022/NUS-IMCB-02, OFIRG21jun-0097, CSASI21jun-0006, MTCIRG21-0071, H22G0a0005, I22D1AG053, HLCA23Feb-0031, SC36/19-000801-A044, H24G1a0015, M24N2K0087, M25P1a0054 and NRF-SFSRND2FS-0005.

Author contributions

All authors researched data for the article. All authors contributed substantially to discussion of the content. All authors wrote the article. R.Y.H.L. and A.K.K.T reviewed and/or edited the manuscript before submission.

Competing interests

A.K.K.T. is a co-founder and shareholder of BetaLife Pte Ltd but is not employed by BetaLife Pte Ltd. The other authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41574-025-01220-x.

Peer review information *Nature Reviews Endocrinology* thanks Guoqiang Gu and the other, anonymous, reviewer for their contribution to the peer review of this work.

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